REMARKS

I. Status Summary

Claims 1-8 are pending in the present U.S. patent application.

The rejection of claims 1-13 and 15-21 under 35 U.S.C. § 103(a) presented in the Official Action of May 21, 2003 has been withdrawn by the United States Patent and Trademark Office (hereinafter "the Patent Office").

Claims 1-8 have been rejected under 35 U.S.C. §102(b) upon the contention that the claims are anticipated by U.S. Patent No. 5,707,807 to Kato (hereinafter "Kato").

Claims 1-8 have been rejected under 35 U.S.C. §102(e) upon the contention that the claims are anticipated by U.S. Patent No. 6,221,600 to MacLeod *et al.* (hereinafter "MacLeod *et al.*").

Claims 1, 5, and 8 have been amended.

Support for the amendment to claim 1 can be found throughout the specification as filed, including particularly on page 2, lines 15-20. Additional support for the amendment can be found page 7, lines 7-19 (the degeneracy in the recognition or cleavage sequence is used to fractionate the restriction fragments through the ligation step), and on page 18, line 12, through page 19, line 7.

The amendment to claim 5 is solely for the purpose of correcting a typographical error and is not to be interpreted as a surrender of any subject matter originally encompassed by the claim.

Support for the amendment to claim 8 can be found throughout the specification as filed, including particularly on page 2, lines 15-20. Additional support for the amendment can be found in the specification as filed on page 6, line 28, through page 7, line 6 ("the ability to accurately quantitate the amount of polynucleotide present"), on page 7, line 20, through page 8, line 9 ("another advantage of the present invention is that it provides an ability to quantitate the results of the methods"), and on page 9, lines 1-16 ("The method provides a further step of detecting the PCR products, preferably by gel electrophoresis, and analyzing for quantitative representation").

Accordingly, no new matter has been added by any of the amendments to the claims. Reconsideration of the application as amended and based on the arguments set forth herein below is respectfully requested.

II. Response to the Anticipation Rejection over Kato

Claims 1-8 have been rejected under 35 U.S.C. §102(b) upon the contention that the claims are anticipated by Kato. The Patent Office asserts that Kato teaches the claimed method.

After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

It is well settled that for a cited reference to qualify as prior art under 35 U.S.C. § 102, each element of the claimed invention must be disclosed within the reference. "It is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention." Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986). With regard to the instant rejection, the Official Action lists on pages 2-3 various elements of the claims that the Patent Office asserts are disclosed by Kato. However, the Patent Office does not provide reference to any disclosure in Kato with regard to element (e) of claim 1. Applicants further respectfully submit that this element is not disclosed at all in Kato, and thus the instant lack of novelty rejection of the claims is improper.

To elaborate, claim 1 recites *inter alia* the following: a method for sequence-specific identification, separation and quantitation of polynucleotide fragments in a population of polynucleotides comprising... (e) detecting and quantifying said polynucleotide fragments (emphases added). Applicants respectfully submit that Kato does not teach quantifying said polynucleotide fragments.

The Patent Office asserts that Kato teaches "quantitation of amplified nucleic acid molecules using a sequencer, which automatically records the size of the fragments based on molecular weight" (Official Action at page 4). Applicants

respectfully submit, however, that the recordation of the sizes of the fragments is not a quantitation as that term is generally understood and as used in the instant application. Applicants respectfully submit that the term "quantitate" is defined by the Merriam-Webster dictionary as "to measure or estimate the quantity of" (emphasis added), and further that this definition is consistent with the use of the term in the instant application but not with the use asserted by the Patent Office.

Turning to the specification of the instant application, applicants respectfully submit that it is clear that one aspect of the claimed methods is that differences in the levels of gene expression can be investigated. This is clearly described throughout the specification, including particularly on page 3, line 19, through page 4, line 8. Additionally, page 25, lines 26-28 specifically recites that "PCR amplification is restricted to <25 cycles in order to achieve the linear representation of the mRNA concentration" (emphasis added). The clear significance of this aspect of the disclosure is that the claimed methods are designed to accurately reflect the relative abundances of the various components of the starting materials in the amplified products, such that quantitative measurements of the products can be used as a proxy for the relative expression levels of the nucleic acids from which the amplified products are derived.

Applicants respectfully submit that Kato does not attempt to quantitate expression levels, and thus does not anticipate this element of claim 1. Applicants further respectfully submit that that this is clear from the disclosure of Kato, which recites that the PCR reactions employed 25-35 cycles of amplification. Applicants respectfully submit that as is known in the art, the number of amplification cycles must be limited to ensure quantitative amplification of targets.

Applicants respectfully submit that claim 1 of the instant application recites amplifying is "no more than 25 cycles". Kato, on the other hand, employs 25-35 cycles. Applicants respectfully submit that one of ordinary skill in the art would recognize that for transcripts that are expressed at higher levels, PCR amplification will be non-representative of transcript abundance at greater than 25 cycles of amplification.

Thus, applicants respectfully submit that the method of Kato cannot be used to perform quantitation of PCR products using 25-35 cycles of amplification and have this number be reflective of the original abundance of a transcript in a starting material relative to the abundances of other transcripts in the starting material. As such, applicants further respectfully submit that Kato does not support the present lack of novelty rejection of claim 1.

Accordingly, applicants respectfully submit that Kato does not support a rejection of claim 1 under 35 U.S.C. §102(b). Claims 2-7 all depend directly or indirectly from claim 1, and thus also include the elements of claim 1. As a result, applicants respectfully request that the rejection of claim 1-8 under 35 U.S.C. §102(b) over Kato be withdrawn, and respectfully solicit a Notice of Allowance to that effect.

III. Response to the Anticipation Rejection over MacLeod et al.

Claims 1-8 have been rejected under 35 U.S.C. §102(e) upon the contention that the claims are anticipated by MacLeod *et al.* The Patent Office asserts that MacLeod *et al.* teaches *inter alia* the following:

(a) reverse transcribing an RNA population to provide a double-stranded cDNA population;

(b) digesting said cDNA population with one or more restriction endonucleases having a degenerate recognition or cleave sequence;

(c) ligating said fragments to a series of adapters lacking recognition sequences, wherein each adapter is cohesive to all possible overhangs; and

(d) amplifying said restriction fragments.

Official Action at pages 4-5.

After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

As noted above, for a cited reference to qualify as prior art under 35 U.S.C. § 102, each and every element of the claimed invention must be disclosed within the reference. Hybritech Inc. v. Monoclonal Antibodies, Inc. Applicants respectfully submit that the Patent Office has not established that claims 1-8 lack novelty over MacLeod *et al.* because the cited reference does not teach each and every element of the claims. In particular, applicants respectfully submit that MacLeod *et al.* does not teach using selective adapter ligation to fractionate the restriction fragments, or PCR designed to amplify only a subset of the ligated restriction fragments. Rather, the only examples presented in MacLeod *et al.* employ the use of immobilized nucleic acids that are cut with restriction enzymes that do not employ degenerate recognition or cleavage sequences. The Patent Office attention is directed to Example 2 of MacLeod *et al.*, in which the enzymes employed are Dpn II and Nla III, each of which recognizes a four-base, non-degenerate sequence: 5'-GATC and 5'-CATG, respectively. Thus, when MacLeod *et al.* attach adapters and perform PCR amplification, all of the fragments that have a Dpn II end and an Nla III end are amplified. Applicants respectfully submit that the combination of linker ligation and amplification thus does not fractionate the restriction fragments.

Applicants further respectfully submit that the methods disclosed in MacLeod *et al.* are designed such that the following occurs. First, the nucleic acid is cut with a first restriction enzyme, a linker corresponding to the overhang of the first restriction enzyme is added, and the nucleic acid is immobilized for further processing. In this way, the disclosed methods produce a pool of restriction fragments that have the recognition site for the first enzyme at the 5' end. Next, a second enzyme is used to liberate the immobilized nucleic acid from the substrate. After the second digestion, linkers corresponding to the second restriction enzyme are added, which results in a population of nucleic acids that has a 5' linker corresponding to the first enzyme and a 3' linker that corresponds to the second enzyme, and a second population that has both 5' and 3' ends that correspond to the second enzyme.

Applicants respectfully submit that the general linker ligation strategy disclosed by MacLeod *et al.* would result in linkers being added to all of the nucleic acid molecules present in the sample. This is in contrast to the method of claim 1, which recites (c) ligating said restriction fragments to a series of adapters lacking restriction endonuclease sites, each adapter having a sequence complementary to one of said overhangs... wherein each ligating reaction is performed with one adapter of said series of adaptors and said one adapter can be ligated to only a subset of said restriction fragments. Support for these elements of claim 1 can be found throughout the specification as filed, including particularly in the claims as originally filed. Additional support can be found on page 6, lines 28-31 (selective ligation of digested DNA with perfectly matching adapter sequence to fractionate DNA fragments into subpools of various sizes), on page 7, lines 10-19 (fractionation into N^m pools), and in Example 5 beginning on page 36, line 18 (see particularly page 40, line 25, in which the BsaJ I adapter is indicated to be "1 of 16 kinds" and page 40, lines 28-29, which state that 16 ligations were performed for each digestion reaction). This difference between the instantly claimed method and MacLeod *et al.* is conceded by the Patent Office on page 5 of the Official Action, wherein the Patent Office asserts that MacLeod *et al.* teaches "(c) ligating said fragments to a series of adapters lacking restriction endonuclease sites... wherein each adapter is cohesive to all possible overhangs" (emphasis added, parenthetical and citations omitted).

Thus, since claim 1 recites ligating an adapter to only a subset of the restriction fragments that have been digested with the one or more enzymes, and the cited MacLeod *et al.* reference teaches the use of linkers that can be ligated to all of the restriction fragments produced, applicants respectfully submit that the cited MacLeod *et al.* reference does not support the instant rejection of claim 1.

Stated another way, claim 1 recites the use of restriction enzymes that generate N^m different single stranded overhangs, and wherein for at least one of said restriction endonucleases N is 2-4 and m is 1-5. Thus, the method of claim 1 generates at least two different single stranded overhangs from at least one of the restriction enzymes.

Applicants respectfully submit that this is not taught by MacLeod *et al.*, and further that MacLeod *et al.* discloses no Examples that employ any enzyme that creates more than one type of single stranded overhang.

The Patent Office asserts that Table 1 of MacLeod *et al.* discloses the use of degenerate restriction enzymes. Applicants respectfully submit that MacLeod *et al.* does not teach the use of individual adapters in separate ligation reactions to fractionate the pool of restriction fragments followed amplification reactions that amplify a subset of said restriction fragments for no more than 25 cycles with a primer comprising a detectable label, wherein said primer is designed to amplify only those restriction fragments to which said one adapter of said series of adapters has been ligated as recited in claim 1. Rather, MacLeod *et al.* discloses the use of linkers that are designed to be ligated to all and not a subset of the restriction fragments that are produced by the digestion of the cDNAs with the first and second enzymes.

Accordingly, applicants respectfully submit that MacLeod *et al.* does not teach each and every element of claim 1. As such, MacLeod *et al.* does not support a rejection of claim 1 under 35 U.S.C. § 102(b). Claims 2-8 all depend directly or indirectly from claim 1, and thus include the elements of claim 1. As a result, applicants respectfully submit that claims 1-8 are condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

CONCLUSIONS

In light of the above Amendments and the Remarks presented hereinabove, it is respectfully submitted that claims 1-8 are in proper condition for allowance, and such action is earnestly solicited.

If any minor issues should remain outstanding after the Examiner has had an opportunity to study the Amendment and Remarks, it is respectfully requested that the Examiner telephone the undersigned attorney so that all such matters may be resolved and the application placed in condition for allowance without the necessity for another Action and/or Amendment.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiencies or credit any overpayments associated with the filing of this correspondence to Deposit Account Number **50-0426**.

Respectfully submitted,
JENKINS, WILSON & TAYLOR, P.A.

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